UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY

FOREST INSECT INVESTIGATIONS

THE RELATION OF UNSEASONAL TEMPERATURES TO BARK-BEETLE MORTALITY

By
James C. Evenden
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and

THE RELATION OF LIPID AND MOISTURE CONTENT TO COLD-HARDINESS OF MOUNTAIN PINE BEETLE LARVAE

By
W. D. Bedard
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Forest Insect Laboratory
Coeur d'Alene, Idaho
June 24, 1938

U. S. GOVERNMENT PRINTING OFFICE: 1928 J 76133

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Forest Insect Laboratory
Coeur d'Alene, Idaho
June 27, 1938

Dr. F. C. Craighead
Washington
D. C.

Dear Dr. Craighead:

There are enclosed two copies of a report "The Relation of Unseasonal Temperatures to Bark-Beetle Mortality" by James C. Evenden and "The Relation of Lipid and Moisture Content to Cold-hardiness of Mountain Pine Beetle Larvae" by W. D. Bedard. The extra copy is for circulation among the eastern stations if you so desire. It is trusted that you will find time to go over this report and let us have your reaction as to the results which have been accomplished and as to further work which we should attempt during the coming season.

The outstanding results indicate that when mountain pine beetles are subjected to normal fall temperatures a resistance is developed which will withstand the lowest temperatures encountered in this region during winter months. This resistance develops quite rapidly in the fall of the year and decreases with apparently the same rapidity with the cessation of winter temperatures. It was also rather definitely established that the normal resistance of overwintering larvae can be broken if they are exposed to activity temperatures, the degree to which this resistance is reduced being dependent upon the length of exposure to activity temperatures. It was also demonstrated that when this resistance has once been broken it can not be completely restored, so that the occurrence of unusually warm weather during the winter season followed by normal low temperatures could produce severe brood mortality.

Trusting that we may have the benefit of your comments and criticisms, I remain

Respectfully yours,

James C. Evenden
Senior Entomologist

cc to Mr. Miller
Mr. Keen
Mr. Beal

DICTATED BY MR. EVENDEN BUT SIGNED
IN HIS ABSENCE TO AVOID DELAY.

THE RELATION OF UNSEASONAL TEMPERATURES TO BARK-BEETLE MORTALITY

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James C. Evenden
Senior Entomologist*

INTRODUCTION

For a number of years it has been recognized that the trend of serious bark-beetle infestations may be materially altered by an abnormal mortality in overwintering broods. As such changes often eliminate the need for the institution of previously planned control projects, the cause of such abnormal mortality must be explained if forest protectionists are to be forewarned of its occurrence and secure the full advantages of the economic benefits offered. Extreme low temperatures were first offered as the direct cause of this condition, and in some instances when abnormally severe they are unquestionably responsible. However, the occurrence of such mortality when minimum temperatures were not in excess of normal conditions requires that other factors be sought in the explanation of this problem.

Though field studies were instituted and attempts made to correlate abnormal brood mortality with available temperature records, the results secured did not offer a satisfactory explanation of the reason for its occurrence. To secure the fundamental data necessary in the solution of this problem a low-temperature cabinet was added to the equipment of the Coeur d'Alene laboratory during the summer of

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*Though the writer in assuming the leadership of these studies is responsible for the planning of the experiments as conducted, credit is extended to Mr. T. T. Terrell who conducted the major portion of the actual work in connection with each operation.

1936. Intensive studies concerning the lethal effects of low temperatures upon overwintering bark-beetle larvae were first instituted at this laboratory in December of that year.

In attacking this project it soon became evident that its fundamentals were quite different than had been anticipated. Furthermore, the minimum temperature possible with the freezing cabinet was found to be insufficient for the successful prosecution of the project in this region. However, the data secured during the first season's work contributed to a rather clear conception of the relation between critical temperatures and bark-beetle mortality.

SUMMARY OF RESULTS SECURED 1936-1937*

In addition to the development of a satisfactory operative technique for the execution of planned experiments, the outstanding facts portrayed by the first season's data are summarized briefly as follows.

1. Larval mortality associated with an exposure to a critical temperature can perhaps be considered as direct and indirect, direct mortality resting in the larvae which are killed by the exposure, with delayed mortality or an arrested physical or sexual development constituting possible indirect effects.

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*Unpublished manuscript -

Evenden, James C., Bedard, W. D., Low Temperatures Critical to Bark-Beetle Larvae; and The Effect of Tempering as a Means of Increasing Cold-Hardiness upon the Lipid and Moisture Content of Mountain Pine Beetle Larvae, July 9, 1937.

2. Presence of a varied cold-hardiness of larvae of the same instar from the same piece of host material.

3. Existence of a relationship between the development of larvae and their resistance to low temperatures, with the first and fifth instar representing the two extremes.

4. Increased larval resistance to low temperatures with the normal advent of cold weather and decreased resistance with the cessation of the winter season.

5. The lag or difference between subcortical and air temperatures being governed by bark thickness and the rate at which the air temperatures change.

6. Nonexistence of a correlation between tree diameters and the difference in subcortical and air temperatures.

7. Freezing of bark-beetle larvae to a solid mass being not always fatal, though indirect effects are still unknown.

8. The existence of a relationship between the resistance of larvae to low temperatures and their body chemistry.

With these potentially established theories as a foundation for subsequent experimentation, the work during the past winter (1937-1938) was planned with the idea of securing those data which will provide a clearer conception of the relationship which exists between temperatures and bark-beetle mortality.

EQUIPMENT

Though the low-temperature cabinet secured in 1936 continued to work fairly satisfactorily, its lowest temperature is ineffective

against the resistance of larvae tempered by normal winter temperatures. The Brown control was used for maintaining a constant set temperature within the cabinet; a potentiometer and thermocouples were used for recording actual temperature within the cabinet. As an added check a calibrated self-recording minimum thermometer was placed in the cabinet, which gave at the end of each exposure the minimum temperature reached.

An electric refrigerator which was added to the equipment of the laboratory proved of value in storing larvae from the time they were removed from their host until they were exposed in the low-temperature cabinet.

A soxhlet extraction apparatus was employed for the purpose of determining the lipid content of larvae in relation to their resistance to low temperature.

OPERATIVE TECHNIQUE

To avoid the repetition of details in the following descriptions the technique employed in connection with all experiments conducted during the past season is discussed under this caption.

1. Logs containing 1937 broods of the mountain pine beetle in white pine were removed from the Coeur d'Alene National Forest in late October and stored under fairly comparable conditions near Burns Summit, approximately 15 miles east of Coeur d'Alene.

2. When needed, these logs were brought to the laboratory, placed upon a piece of canvas, and the bark carefully removed. The

log was then removed from the canvas and the larvae rolled gently together by lifting the corners of the canvas. This procedure mixed the larvae from all portions of the logs and provided a fairer sample. If more than one log were peeled, the larvae were all mixed together so as to avoid existing variations between logs.

3. Larvae were then placed in petri dishes filled with blackened paraffin, in which 50 small holes had been drilled. The process of removing the larvae from the logs to storage in the refrigerator was performed as quickly as possible, and under proper temperature conditions, so as to avoid exposures to conditions which might tend to break the existing degree of resistance to low temperatures.

4. Petri dishes of larvae were stored in an electric refrigerator at a constant temperature of 38°F - 40°F until used. A small piece of saturated blotting paper ½ inch square was placed in the lid of each dish.

5. Exposures in the low-temperature cabinet at stated temperatures were in the covered petri dishes for 2 hours and 15 minutes unless otherwise stated.

6. After exposure the petri dishes were placed in a water-proof petri dish holder set in a covered "Kool Kan" filled with ice. After 8 hours the holders of petri dishes were placed in a bucket of ice, which was allowed to melt and the water to gradually assume a room temperature.

7. Petri dishes of exposed larvae were examined at 24-, 48-, and 72-hour periods. At the time of each examination all living larvae

were removed.

8. In connection with each test of larval resistance to low temperatures, the lipid content of a sample of larvae was secured.

EXPERIMENTS CONDUCTED DURING 1937-1938 SEASON

Though for the most part the technique employed in the execution of the following experiments had been previously developed, additional tests were necessary to determine the proper use of the storage refrigerator which had been added to the equipment of the laboratory. As a result the experiments of the past season are separated into "operative technique" and "low temperature resistance studies".

OPERATIVE TECHNIQUE

Technique Experiment A

<u>Objective</u> - Mortality associated with larvae stored at 38°F - 40°F in refrigerator during period required for the execution of a low-temperature test (2-3 days).

<u>Insect</u> - Mountain pine beetle.

<u>Host</u> - Western white pine.

<u>Locality</u> - Coeur d'Alene National Forest.

<u>Date</u> - Started November 29, 1937

<u>Description of Experiment</u> - Moisture being considered as the fundamental factor governing the mortality of stored larvae at below activity temperatures, the set-up of this experiment was as follows:

Test No. I - Eight petri dishes of 50 larvae each with an open vessel of water were placed in a covered container which was held in the refrigerator.

A - Four dishes covered.

B - Four dishes uncovered.

Test No. II- Eight covered petri dishes of 50 larvae each were placed on refrigerator shelf.

A - Four dishes had small ($\frac{1}{2}$-inch-square) bits of saturated blotting paper fastened to inside of lid.

B - Four dishes had no blotting paper.

The results of these tests, which were carried far beyond the original limits of the experiments, are shown in the following table.

Table I

MORTALITY OF MOUNTAIN PINE BEETLE LARVAE
STORED IN REFRIGERATOR
38°F. - 40°F.

Test	Dish	Percent mortality after 5 Days	10 Days	15 Days	30 Days	60 Days	109 Days	161 Days	Remarks
Covered petri dishes in covered container containing an open vessel of water									
IA	1	0	0	0	0	0	6	20	Larvae remained in fair condition. After 100 days considerable mold developed in these dishes.
	2	0	0	0	0	0	0	4	
	3	0	0	0	Discontinued after 15 days, as petri dishes were required for other experiments.				
	4	0	0	0					
Uncovered petri dishes in covered container containing an open vessel of water									
IB	1	0	0	0	0	0	0	18	Larvae remained in excellent condition. Mortality is believed to have resulted from a condensation of moisture which filled some of the larval cells.
	2	0	0	0	0	0	0	32	
	3	0	0	0	Discontinued after 15 days.				
	4	0	0	0					

Table I (Cont.)

Test	Dish	Percent mortality after 5 Days	10 Days	15 Days	30 Days	60 Days	109 Days	161 Days	Remarks
Covered petri dishes on refrigerator shelf with small piece of saturated blotting paper in cover									
									Though there was little
	1	0	0	0	Discontinued				mortality in this test,
IIA	2	0	0	0	0	0	0		the larvae did not appear
	3	0	0	0	0	0	4		to be in as good condition
	4	0	0	0	Discontinued				as those in I. Larvae were
									decidedly drier and were
									quite yellowish in color.
Covered petri dishes on refrigerator shelf with no added moisture.									
									Higher mortality and those
	1	0	0	0	Discontinued				surviving at 109 days were
IIB	2	0	0	0	6	14	70		quite dry and yellowish
	3	0	0	0	4	26	78		in color.
	4	0	0	0	Discontinued				

The preceding data indicate that moisture is the factor governing mortality of bark-beetle larvae when stored at below-activity temperatures. Though in test IIB no mortality occurred during the first ten days of storage, it is evident that this treatment was not satisfactory and should be avoided, as undoubtedly a slight desiccation would affect larval resistance to subsequent low temperatures. Any of the other three treatments would seem to be satisfactory for the purpose of storing larvae for a short period, with the placing of saturated blotting paper in the petri dish lids as the simplest procedure. If methods were employed to prevent a condensation of moisture within the petri dishes, it is believed that larvae, if placed in uncovered petri dishes within a covered container holding an open vessel of water, could be stored for a longer period than indicated in this experiment.

Technique Experiment B

Objective - To determine if the resistance of bark-beetle larvae to low

temperatures is changed by storing for a period of ten days in a refrigerator at a temperature of 38°F - 40°F.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - Started November 10, 1937.

Description of Experiment - Sixteen petri dishes of larvae were placed in the refrigerator. Eight of these dishes were exposed separately to a temperature of -10°F. for 2 hours and 15 minutes after different intervals of storage. The remaining eight dishes were used as checks for storage mortality. It is regretted that at the time this experiment was conducted the larvae were stored in covered petri dishes with no provision for adding moisture. As a result there was considerable mortality in the checks, and it is possible that some of that which occurred in the exposed dishes must be credited to storage. However, subsequent experiments indicate that there would have been very little if any mortality during the first few days of this experiment. The data secured are presented in the following table.

Table II

EFFECTS OF STORAGE IN REFRIGERATOR UPON LARVAL RESISTANCE TO LOW TEMPERATURES

Date exposed	Dish	Temperature	Hours of storage	Percent mortality
Nov. 12, 1937	1	-10°F.	48	36
Nov. 16, 1937	2	"	144	30
Nov. 17, 1937	3	"	168	48
Nov. 24, 1937	4	"	336	62
" " "	5	"	"	62
" " "	6	"	"	66
" " "	7	"	"	42
" " "	8	"	"	40
Examined	Unexposed checks held in refrigerator			
Nov. 24, 1937	9	+38°F.	336	16
	10	"	"	12
	11	"	"	16
	12	"	"	14
Nov. 26, 1937	13	"	384	40
	14	"	"	46
	15	"	"	42
	16	"	"	42

The data in the preceding table indicate a decreased resistance to low ~~larval~~ temperatures after storage for 144 hours, while prior to that time there was no apparent reduction in larval resistance. However, the increased mortality after the 144 hours of storage undoubtedly rests in the refrigerator mortality as a result of improper treatment prior to exposure, as indicated by the controls and as shown by Experiment A. The conclusion drawn from this experiment and supported by subsequent tests is that storage in a refrigerator at below-activity temperature has no effect upon the cold-hardiness or resistance of bark-beetle larvae to low temperatures.

Technique Experiment C

Objective - Proper treatment of larvae subsequent to exposure at a set

temperature.

<u>Insect</u> - Mountain pine beetle.

<u>Host</u> - Western white pine.

<u>Locality</u> - Coeur d'Alene National Forest.

<u>Date</u> - November 29, 1937.

<u>Description of Experiment</u> - The method of treating larvae after being exposed to low temperatures has been described under "operative technique". In this method the exposed dishes of larvae were placed in a water-proof petri dish holder and set in a "Kool Kan" filled with ice. After 8 hours the petri dish holders were placed in a bucket of ice, which was allowed to melt and the water to assume room temperature. Though this procedure was considered as entirely satisfactory, the possibility of using the storage refrigerator for holding larvae after exposure prompted the following tests.

I. Two petri dishes of larvae exposed at temperatures ranging from +10°F. to -25°F. Following each exposure

A. One dish was placed in ice storage in manner previously employed.

B. One dish placed below ice compartment in storage refrigerator for 6-8 hours, then removed to bottom of refrigerator box.

The results of this experiment are shown in the following table.

Table III

MORTALITY OF EXPOSED LARVAE UNDER TWO METHODS
OF SUBSEQUENT TREATMENT

Date	Dish	Temp. F.	Mortality	Remarks
Placed in ice storage after an exposure of 2 hours and 15 minutes				
Nov. 29	1	+10	0	
"	2	+ 5	0	
Nov. 30	3	0	0	
"	4	- 6	0	
Dec. 1	5	-10.6	4%	
"	6	-15	18%	This variation undoubtedly due to the presence of a few nonresistant larvae.
"	7	-20	6%	
Dec. 2	8	-25	16%	This would seem to be the normal mortality which could be expected at this period of resistance development.
Placed in storage refrigerator after an exposure of 2 hrs. and 15 min.				
Nov. 29	1A	+10	0	
"	2A	+ 5	0	
Nov. 30	3A	0	0	
"	4A	- 6	2%	This mortality was undoubtedly due to an injured larvae.
Dec. 1	5A	-10.6	0	
"	6A	-15	4%	
"	7A	-20	6%	
Dec. 2	8A	-25	34%	Could be normal mortality, though considerably higher than that secured in dish # 8.

Though the data presented in table III show a higher mortality in the larvae exposed to -25°F. and stored in the refrigerator, there did not seem to be a great deal of difference in the two treatments. However, it is entirely possible that some of the 34% mortality secured in dish 8A could have resulted from sample variation, as the increase from 6% mortality at -20°F. to 34% at -25°F. would seem to be too severe.

As these data were somewhat confusing, another test of these two methods of treating exposed larvae was made on December 8. The results of this test are shown in table IV.

Table IV

MORTALITY OF EXPOSED LARVAE UNDER TWO METHODS OF SUBSEQUENT TREATMENT

Date	Dish	Temp. F.	Mortality	Remarks
Placed in refrigerator after an exposure of 2 hours and 15 minutes				
Dec. 8	1	-2	0	
"	2	-6.5	0	
"	3	-11.5	4%	Undoubtedly due to the presence of 2 nonresistant or injured larvae.
Dec. 9	4	-15.5	0	
"	5	-21	0	
Dec. 10	6	-25	2%	
Placed in ice storage after an exposure of 2 hours and 15 minutes				
Dec. 8	3A	-11.5	2%	Undoubtedly due to the presence of 1 nonresistant or injured larva.
Dec. 9	4A	-15.5	0	
"	5A	-21	0	
Dec. 10	6A	-25	0	

The results of this test are not very conclusive, as but very little mortality was secured. The slight mortality secured at -11.5°F. in both treatments and at -25°F. in the refrigerator-stored larvae is assumed to be the result of injured or weakened larvae. Here again there did not seem to be a great deal of difference in the results of the two treatments.

As a further test of these two methods of treating exposed larvae a variation of the ones previously conducted was started on December 28. Sixteen dishes of larvae were placed in the tank at -20°F. Two dishes were removed at the end of periods varying from 2 hours to 4 days, with one dish being placed in the refrigerator and the other in ice storage. The results of this test are shown in table V.

Table V

MORTALITY OF EXPOSED LARVAE UNDER TWO METHODS OF SUBSEQUENT TREATMENT AFTER DIFFERENT LENGTH EXPOSURES TO A TEMPERATURE OF -20°F.

Date	Dish	Exposure	Mortality	Remarks
		Dishes placed in ice storage after exposure		
Dec. 28	1	2 hrs.	6%	It is difficult to account for this
"	2	4 hrs.	0	mortality at the end of the two-hour
"	3	8 hrs.	0	exposure, though the same data were
Dec. 29	4	16 hrs.	4%	recorded for dish 1A. The answer is
"	5	1 day	6%	believed to rest in the presence of a
	6	2 days	6%	few (3) nonresistant larvae, and it
	7	3 days	4%	would seem that there were about the
	8	4 days	8%	same number in all the dishes with
				the exception of #2 and #3.
		Dishes placed in storage refrigerator after exposure		
Dec. 28	1A	2 hrs.	6%	
	2A	4 hrs.	0	
	3A	8 hrs.	0	
Dec. 29	4A	16 hrs.	8%	
	5A	1 day	20%	These mortality figures are higher than
	6A	2 days	14%	indicated by other tests and are be-
	7A	3 days	12%	lieved to be the result of the treat-
	8A	4 days	12%	ment to which the exposed larvae were
				subjected.

From the data shown in tables III, IV, and V the method of placing the exposed larvae in ice storage, which permits a gradual warming up to a near room temperature, would seem to be the best treatment, and its use was continued in all subsequent experiments.

Technique Experiment D

Objective - To determine the period of exposure necessary to secure maximum effects of a specific temperature.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - March 25, 1938.

Description of Experiment - As prolonged exposures for several days resulted in no greater larval mortality than the 2¼-hour period used in all experiments, this preliminary test was planned to determine if a shorter exposure would be equally effective. To secure these data two groups of larvae were used, one being exposed for 1¼ hours and the other for 2¼ hours. The temperature selected was one which was assumed would provide a partial mortality. The results of these tests are shown in table VI.

Table VI

LARVAL MORTALITY RESULTING FROM EXPOSURES
OF 1¼ AND 2¼ HOURS

Dish	Temp. F.	Mortality	Remarks
			Exposure 1 hour 15 minutes
1	-6	36%	Average mortality for this group of
2	"	46%	250 larvae was 39.6%. Mortality in
3	"	42%	the individual dishes was sufficiently
4	"	30%	close as to indicate representative
5	"	44%	samples.
			Exposure 2 hours 15 minutes
6	-6	34%	Average mortality for this group of 200
7	"	38%	larvae was 33% or 6% lower than the
8	"	26%	shorter exposure. This difference can
9	"	34%	not be considered as significant of the
			two treatments.

The results secured from this test must be regarded as of a preliminary character only, as a series of different-length exposures will be necessary before positive conclusions can be drawn. However, they do indicate that there was practically no difference in the mortality secured from 1¼- and 2¼-hour exposures. Though it is believed that the shorter period would result in equally effective data, as the

longer exposure had been used in all previous experiments, its use was continued throughout the remainder of the season.

Technique Experiment H

<u>Objective</u> - Method of determining larval mortality following an exposure to low temperatures.

<u>Insect</u> - Mountain pine beetle.

<u>Host</u> - Western white pine.

<u>Locality</u> - Coeur d'Alene National Forest.

<u>Date</u> - January 19, 1938.

<u>Description of Experiment</u> - Considerable difficulty is always experienced in determining border-line larval mortality following an exposure to a specific temperature. Some larvae are motionless and show no response to physical disturbance. Some of these "no motion" larvae recover, while others finally succumb. The method believed to answer this problem most satisfactorily is to examine the exposed larvae at 24-, 48-, and 72-hour periods. Though active larvae can be seen with the naked eye, the 48- and 72-hour examinations are always made with a binocular microscope. By removing the living larvae at each examination the chance of increased mortality due to abnormal treatment after exposure is materially reduced.

A question arose as to the necessity of the 24- and 48-hour examinations, and that perhaps the best procedure would be to have the 72-hour examination only. To answer this question three groups of 250 larvae each were placed in the low-temperature cabinet at a temperature of -25°F. and held for 72 hours. At the end of this

period they were all placed in ice storage. All three groups of larvae were treated in the same manner, except for the time of examination. The data secured are shown in the following table.

Table VII

MORTALITY OF LARVAE EXPOSED TO A SPECIFIC TEMPERATURE
AS INDICATED BY DIFFERENT EXAMINATIONS

Dish	Temp. F.	Exposure	% Mortality 24 hrs.	% Mortality 48 hrs.	% Mortality 72 hrs.	Average mortality	Remarks
1	-25°	3 days	80	4	2	3.2%	It will be seen that at the end of the 24-hour examination 63.2% of the larvae were motionless. Of this number 60% subsequently became active.
2	"	"	66	4	4		
3	"	"	66	4	0		
4	"	"	60	8	8		
5	"	"	44	2	2		
6	"	"		2	2	5.2%	
7	"	"		8	4		
8	"	"		6	6		
9	"	"		6	6		
10	"	"		8	8		
11	"	"			4	5.6%	It is possible that there were some low-resistant larvae in dish 13, though in dish 4 there was a final mortality of 8%.
12	"	"			2		
13	"	"			10		
14	"	"			6		
15	"	"			6		

As the 24-, 48-, and 72-hour examinations showed the lowest larval mortality, it would seem, if this one test can be considered as conclusive, that this method of examination arrives at the most satisfactory figure.

LOW TEMPERATURE RESISTANCE STUDIES

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RESISTANCE EXPERIMENT I

Objective - Seasonal changes in the resistance of bark-beetle larvae to extreme low temperatures.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - September 1937 - May 1938.

Description of experiment - Tests conducted during the 1936-37 season indicated a relationship between normal seasonal temperatures and the resistance of bark-beetle larvae to low temperatures. The following experiment was planned to provide those data which would portray the seasonal development of larval resistance to low temperatures. To secure these data tests were conducted at 15-day intervals from September 18, 1937 to June 1, 1938, with the exception of December, January, and February, as during this period the larval resistance was higher than the minimum temperature available with present equipment. In each test 50 larvae were exposed for 2 hours and 15 minutes at a constant temperature, the range of exposures being sufficient to include both extremes of mortality. It is regretted that the present equipment would not permit the point of maximum larval resistance to low temperatures to be established; however, the data secured show quite conclusively the trend of the so-called tempering processes. It is trusted that an inexpensive auxillary unit can be added to the present equipment, which will permit the full completion of this and other experiments.

The data secured are presented graphically in Charts I and II. In the first chart the larval resistance of the individual tests are shown, while in the other, zero, 50% and 100% mortality curves are shown

CHART I

TEMPERATURES LETHAL TO MOUNTAIN PINE BEETLE LARVAE IN RELATION TO SEASONAL RESISTANCE

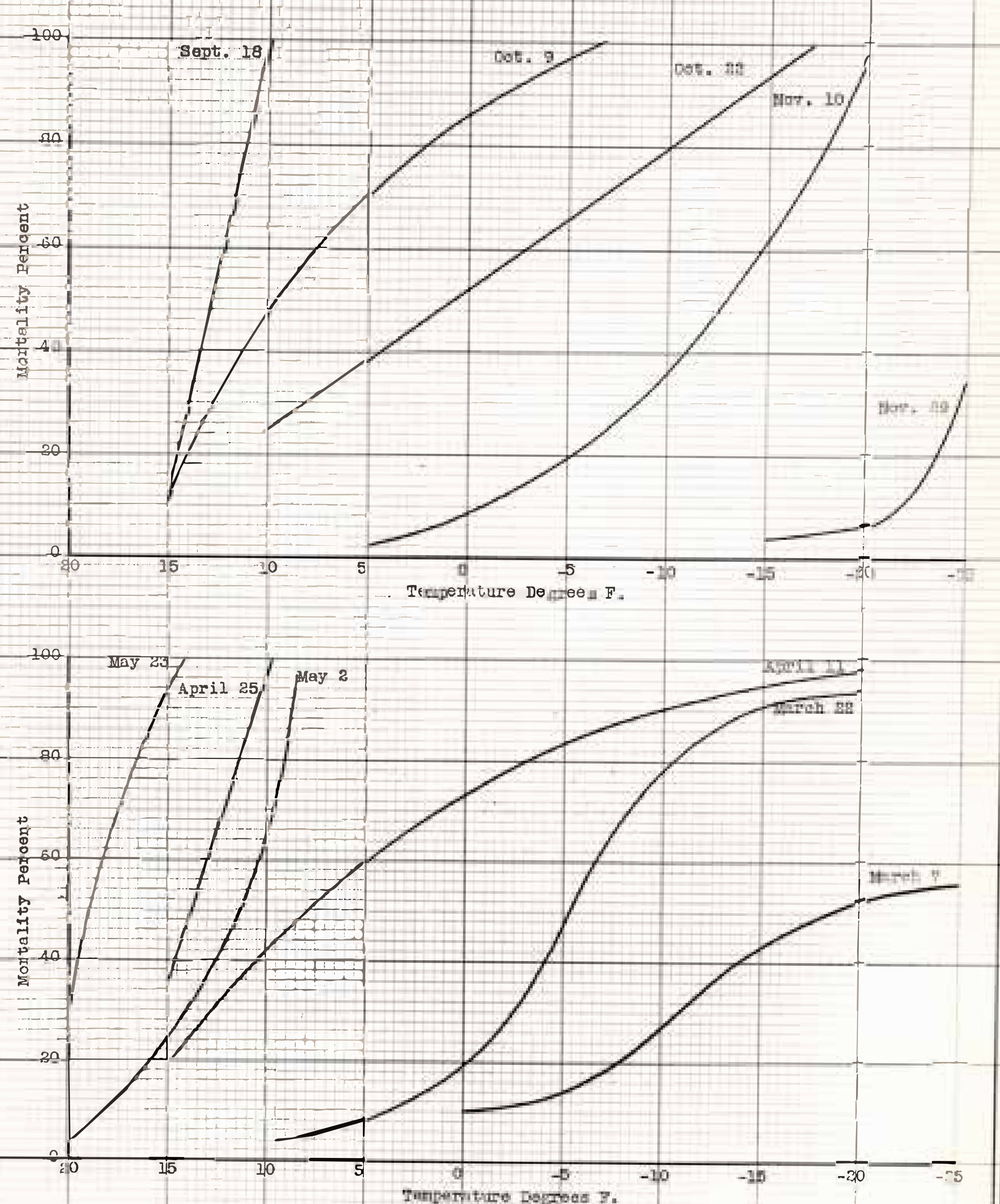
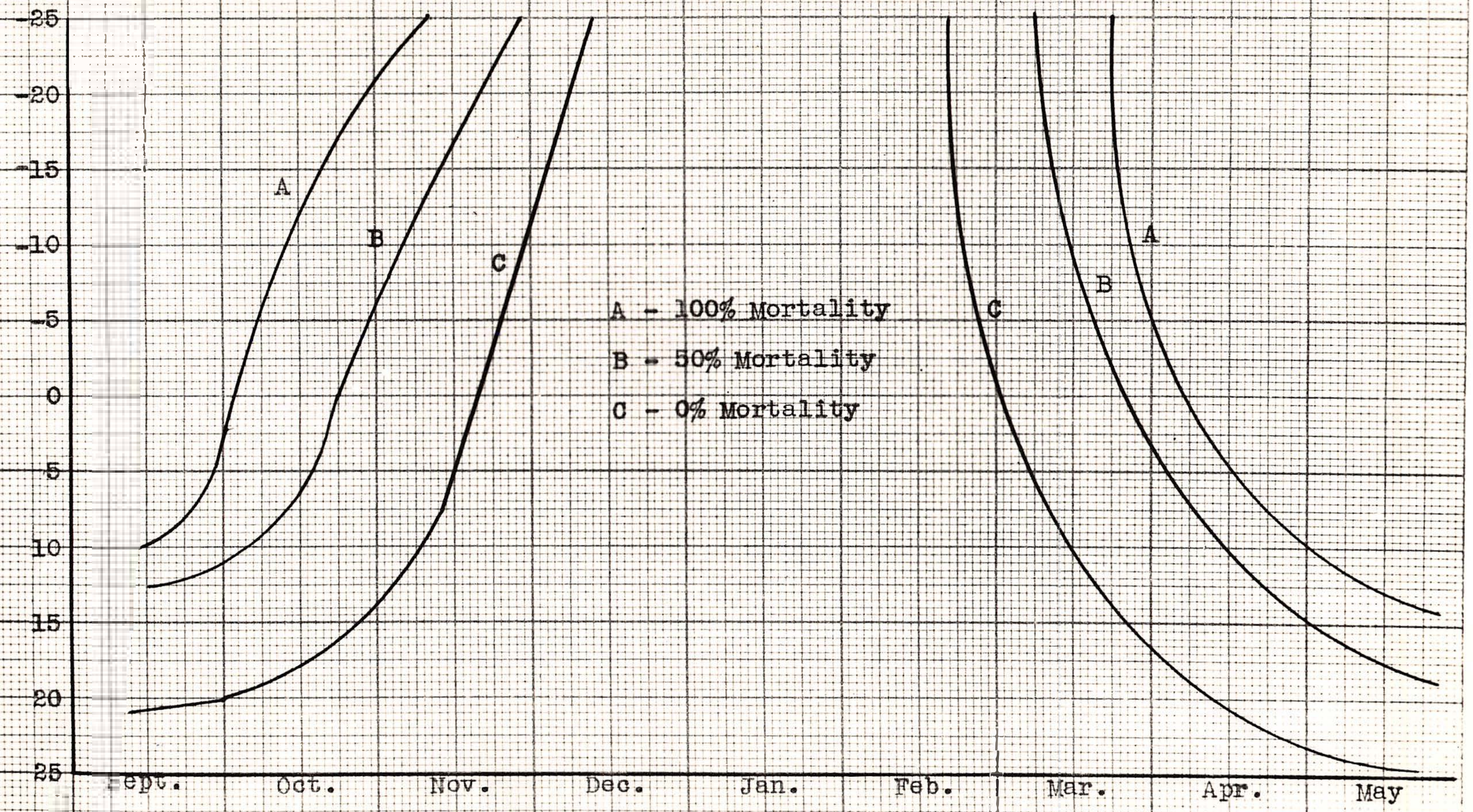


CHART II

SEASONAL CHANGES IN THE RESISTANCE OF
MOUNTAIN PINE BEETLE LARVAE TO LOW TEMPERATURES



for the 1937-1938 season.

These curves can only be accepted as showing the seasonal resistance of mountain pine beetle larvae for this locality during the past winter. Variations in normal temperatures during different years will prevent the adoption of any set of data as indicating the cold-hardiness of larvae for any season. However, they do indicate the interrelationship of seasonal changes in normal temperatures and the tempering process of bark-beetle larvae.

An interesting departure from these data occurred in a test run conducted on May 31. Larvae were in a prepupal stage with a large percentage of the brood having already pupated. Instead of the lowered resistance shown in the test conducted on May 23, conditions were somewhat reversed, and sufficient material was not available to continue the test to a 100% mortality mark. The data from this test are shown in comparison with the May 23 experiment in the following tabulation.

Temperature	May 23 mortality	May 31 Percent frozen	May 31 Mortality
25°F.	0	0	4%
20°F.	22%	20%	14%
15°F.	92%	46%	44%
10°F.	100%	64%	62%

The May 31 experiment demonstrates that the prepupal larvae are more resistant to cold than those which have not reached this stage in their development. These data show a rather close relationship between the number of larvae frozen when removed from the cabinet and the final mortality. After being exposed, a number of the

larvae pupated between the time of being removed from the cabinet and the 48-hour examination, indicating that the exposure had little effect upon subsequent development.

RESISTANCE EXPERIMENT II

Objective - To determine the effects of abnormal warm temperatures upon established cold-hardiness of bark-beetle larvae.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - January 31 - March 8, 1938.

Description of Experiment - This experiment was instituted to determine if a period of unseasonal warm weather would break the low-temperature resistance of overwintering larvae to an extent that mortality would follow the return-to-normal seasonal temperatures. To secure these data it was necessary to subject the larvae within the stored logs to activity temperatures to break the established larval resistance. As this experiment was intended to depict possibilities only, no effort was made to control the activity temperatures to which the larvae were subjected. Infested logs were brought into the laboratory and stored for different-length periods at day and night temperatures varying from 70 to 45 degrees F. After such exposures some logs were returned to the log cache to determine if the normal resistance would be restored, while the larvae were removed from others for low-temperature tests. As each group of logs was brought into the laboratory for exposure to

above-activity temperatures, a test of the larvae was made immediately to determine normal resistance.

TEST IIA

Three infested logs of the same size and comparable brood development were brought into the laboratory on January 31, 1938. A test of maximum resistance showed no larval mortality at -25°F. On February 7 the larvae were removed from one log (A1) and the low-temperature resistance determined. On the same date the other logs (A2 and A3) were returned to the log cache to determine if normal resistance would be restored. These two logs were held at normal winter temperatures until tested for resistance on February 10 (A2) and February 21 (A3).

The results of these tests are shown in Chart III. It will be seen that a 7-day exposure to laboratory temperatures broke the normal resistance of 0% mortality at -25°F. to 100% mortality at -4°F. Furthermore, 90% mortality was reached at +10°F., with only a few more resistant larvae withstanding the lower temperatures. The larvae in logs A2 and A3 responded somewhat to return to normal winter temperatures by showing a slightly increased resistance. These data are protected by tests of normally tempered larvae which showed 0% mortality at -25°F. on Feb. 10 and 21.

TEST IIB

Three infested logs of approximately the same size and comparable brood conditions were stored at laboratory temperatures from

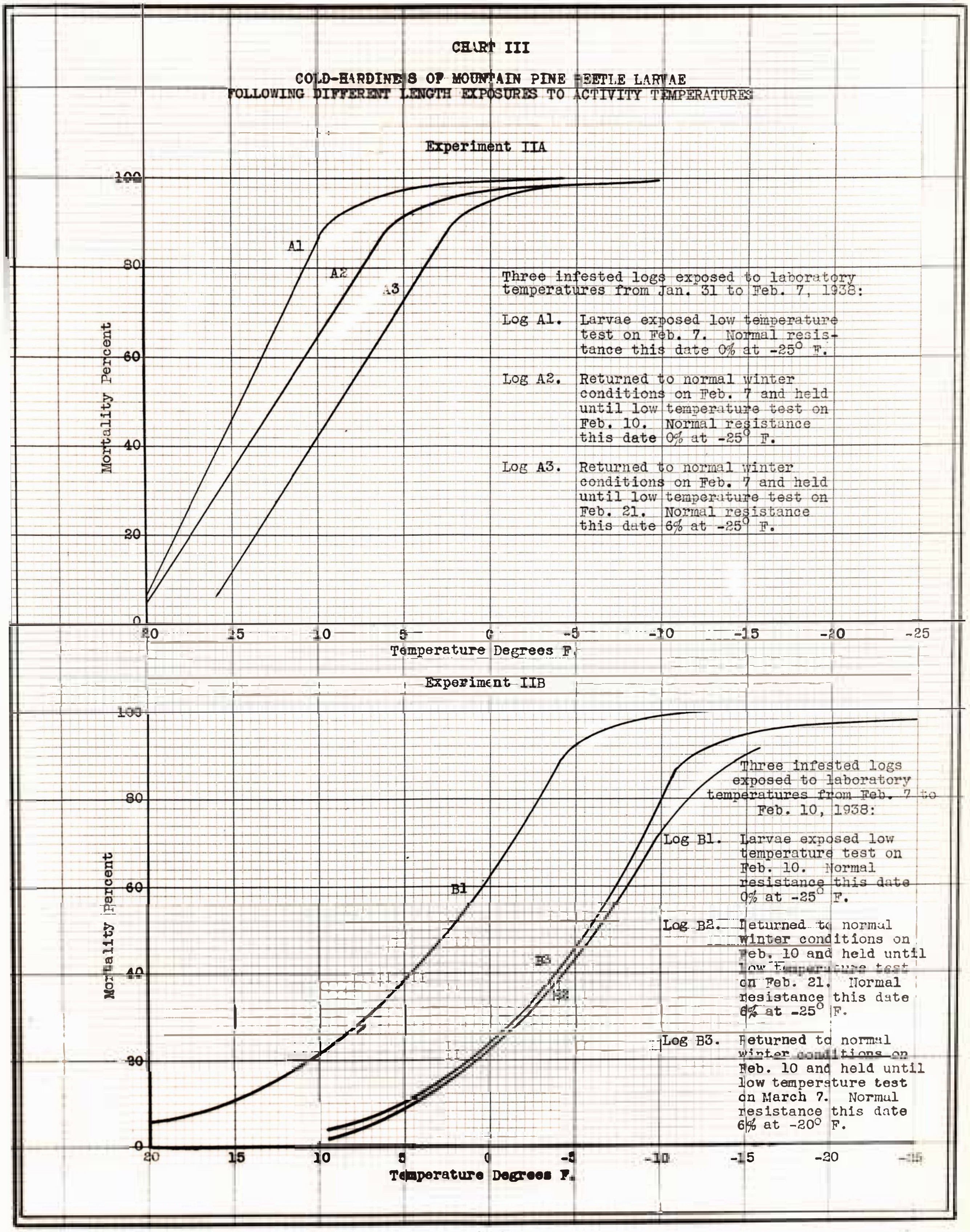


CHART III
COLD-HARDINESS OF MOUNTAIN PINE BEETLE LARVAE
FOLLOWING DIFFERENT LENGTH EXPOSURES TO ACTIVITY TEMPERATURES
Experiment IIA
Mortality Percent
Temperature Degrees F.
A1
A2
A3
Three infested logs exposed to laboratory temperatures from Jan. 31 to Feb. 7, 1938:
Log A1. Larvae exposed low temperature test on Feb. 7. Normal resistance this date 0% at -25° F.
Log A2. Returned to normal winter conditions on Feb. 7 and held until low temperature test on Feb. 10. Normal resistance this date 0% at -25° F.
Log A3. Returned to normal winter conditions on Feb. 7 and held until low temperature test on Feb. 21. Normal resistance this date 6% at -25° F.
Experiment IIB
Mortality Percent
Temperature Degrees F.
B1
B3
B2
Three infested logs exposed to laboratory temperatures from Feb. 7 to Feb. 10, 1938:
Log B1. Larvae exposed low temperature test on Feb. 10. Normal resistance this date 0% at -25° F.
Log B2. Returned to normal winter conditions on Feb. 10 and held until low temperature test on Feb. 21. Normal resistance this date 6% at -25° F.
Log B3. Returned to normal winter conditions on Feb. 10 and held until low temperature test on March 7. Normal resistance this date 6% at -20° F.

Feb. 7, 1938 to Feb. 10, 1938. A test of the maximum resistance of this material on Feb. 7 showed 0% mortality at -25°F. The larvae were removed from log B1 on Feb. 10, and the low-temperature resistance determined. Logs B2 and B3 were returned to the log cache and held until Feb. 21 (B2) and March 7 (B3), when resistance tests were conducted.

The results of these tests are shown in Chart III. Though the three-day exposure to laboratory temperature broke the normal resistance to where 6% mortality occurred at +20°F. and 100% at -13.5°F., it is obvious that the larvae were more resistant than those exposed for seven days (Test IIA). Logs B2 and B3 showed an increased resistance following their return to normal winter temperatures, with a bit more resistance from log B2 on Feb. 21 than from B3 on March 7. This is explained by the fact that on March 7 the normal resistance had decreased somewhat due to warmer weather, with the normal mortality being 56% at -25°F.

TEST IIC

Two infested logs were exposed to laboratory temperatures from Feb. 19 to 21, 1938. Normal resistance of this material showed 6% mortality at -20°F. On February 21 the larvae were removed from C1 and subjected to low temperatures. Log C2 was returned to normal winter condition on the 21st and held until March 7, when it was returned to the laboratory for larval-resistance tests. The results of these tests are shown in Chart IV. It will be seen that the 48-hour exposure to activity temperature reduced the normal resistance to a

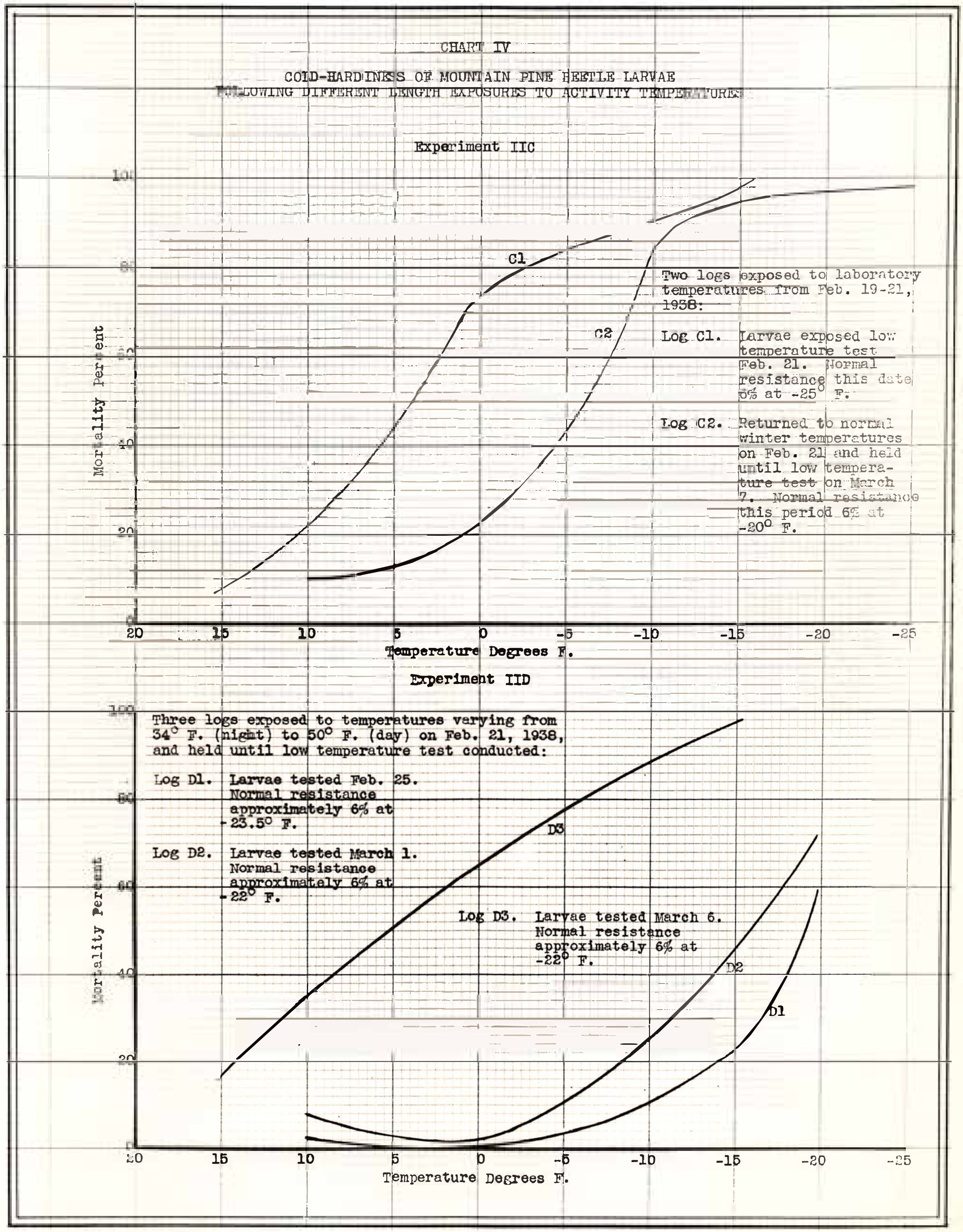


CHART IV
COLD-HARDINESS OF MOUNTAIN PINE BEETLE LARVAE
FOLLOWING DIFFERENT LENGTH EXPOSURES TO ACTIVITY TEMPERATURES
Experiment IIC
Mortality Percent
C1
C2
Two logs exposed to laboratory temperatures from Feb. 19-21, 1938:
Log C1. Larvae exposed low temperature test Feb. 21. Normal resistance this date 5% at -25° F.
Log C2. Returned to normal winter temperatures on Feb. 21 and held until low temperature test on March 7. Normal resistance this period 6% at -20° F.
20 15 10 5 0 -5 -10 -15 -20 -25
Temperature Degrees F.
Experiment IID
Mortality Percent
Three logs exposed to temperatures varying from 34° F. (night) to 50° F. (day) on Feb. 21, 1938, and held until low temperature test conducted:
Log D1. Larvae tested Feb. 25. Normal resistance approximately 6% at -23.5° F.
Log D2. Larvae tested March 1. Normal resistance approximately 6% at -22° F.
Log D3. Larvae tested March 6. Normal resistance approximately 6% at -22° F.
D3
D2
D1
20 15 10 5 0 -5 -10 -15 -20 -25
Temperature Degrees F.

point where an 8% mortality was secured at $15^{o}F$. and 98% at $-15^{o}F$. The larvae from log C2 showed increased resistance as a result of being returned to normal winter temperatures, though they did not return to normal larval resistance for March 7, which was determined as 56% mortality at $-25^{o}F$.

TEST IID

Three infested logs were removed from log cache and exposed to temperatures varying from $34^{o}F$. to $50^{o}F$. Low-temperature-resistance tests were made of the larvae from the logs at 4-(D1), 9-(D2), and 14-(D3) day intervals. The results of this test are shown on Chart IV.

A reduced low-temperature resistance followed the increased time of storage, with a marked difference between the 9- and 14-day periods. The slight mortality at $10^{o}F$. as shown in D1 and D2 no doubt resulted from a few larvae exposed by woodpecker work, which warmed more rapidly than the others and were less resistant.

SUMMARY OF EXPERIMENT II

As a result of these tests it is evident that the normal low-temperature resistance of larvae can be broken easily by exposing the tempered material to so-called activity temperatures. Furthermore, it is evident that the degree to which this resistance is broken is determined by the length and severity of the exposure.

These facts offer the possibility of unseasonal warm weather being equally destructive to overwintering larvae as low temperatures, heretofore believed to have been entirely responsible for winter

mortality. Upon the return to normal winter conditions, following a period of warm weather, lethal temperatures might be encountered which otherwise would have had no effect upon the resistant larvae. It is believed that this possibility may answer for the occurrence of winter mortality during seasons of normal low temperatures.

RESISTANCE EXPERIMENT III

Objective - To determine if prolonged exposures to low temperatures would be more effective against established resistance than the 2½-hour exposure used in all experiments.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - Dec. 27, 1937 and Jan. 7, 1938.

Description of Experiment - There are a few instances to be found in the United States Weather Bureau records for the northern Rocky Mountains where periods of low temperatures have existed with but little difference in the maximum and minimum temperatures. Believing that such condition would have a greater lethal effect upon overwintering bark-beetle larvae than where the maximum temperature rose well above the minimum reading, this experiment was planned in the hope of contributing some more specific information. To secure these data a number of dishes of larvae were placed in the low-temperature cabinet at the same time, with one dish removed after a definite lapsed period of time. The first test was made at -20°F.; however, as the mortality secured was so irregular, a subsequent run was made at -25°F. in the hope of securing more positive

results. The data secured from these two tests are shown in tables VIII and IX.

Table VIII

MORTALITY OF MOUNTAIN PINE BEETLE LARVAE FOLLOWING PROLONGED EXPOSURES TO A TEMPERATURE OF -20°F.

Dish	Length of exposure	Percent frozen at end of exposure	Percent mortality
1	2 hrs.	42	2
2	4 "	62	6
3	8 "	80	6
4	16 "	70	4
5	1 day	40	2
6	2 days	96	4
7	3 "	66	2
8	4 "	24	4
9	5 "	34	6
10	6 "	24	8
11	7 "	20	6
11	8 "	--	7.5

Table IX

MORTALITY OF MOUNTAIN PINE BEETLE LARVAE FOLLOWING PROLONGED EXPOSURES TO A TEMPERATURE OF -25°F.

Dish	Length of exposure	Percent frozen at end of exposure	Percent mortality
1	2 hrs.	22	6
2	4 "	46	0
3	8 "	74	0
4	16 "	84	4
5	1 day	40	6
6	2 days	84	6
7	3 "	92	4
8	4 "	24	8
9	5 "	12	4
10	6 "	26	4
11	7 "	34	12
12	8 "	22	10
13	9 "	32	4
14	10 "	4	8

The data secured from these experiments showed no increased larval mortality as a result of the prolonged exposure. As a result of this test and the Technique Experiment D, where the 1½-hours exposure gave the same results as the 2½-hours used with all experiments, it would seem that mortality follows a very short exposure to a lethal temperature. The mortality shown in tables VIII and IX was unquestionably confined to the less-resistant larvae which are to be found in all samples. It is believed that all of the dishes could have been exposed for the maximum period of exposure, with no greater mortality.

In presenting these data the percent of the larvae which were frozen solid at the end of the exposure has been shown in the tables. Frozen larvae can be very easily detected by their color. It will be seen that there is no relationship between the percent frozen and the subsequent mortality. However, when 100% of the larvae are frozen, the mortality will be between 90 and 100%.

RESISTANCE EXPERIMENT IV

Objective - To determine the effects of an exposure to low temperature upon subsequent larval development.

Insect - Mountain pine beetle.

Host - Western white pine.

Locality - Coeur d'Alene National Forest.

Date - Feb. 11-26, 1938.

Description of Experiment - It is recognized that the technique employed

in connection with this experiment was not above considerable criticism. However, it was planned with the intention of showing possibilities rather than in an attempt to determine the indirect effects of low-temperature exposures. To attempt the study of indirect effects would require more equipment and time than was available for this purpose. In making these simple tests the living larvae from three different exposures were transferred to other petri dishes and held at room temperatures in a covered vessel containing water. The results of this test are shown in table X.

Table X

SUBSEQUENT DEVELOPMENT OF LARVAE AFTER EXPOSURE TO LETHAL LOW TEMPERATURES

Dish	Number of larvae alive after exposure: Feb. 11, 1938	Brood status Feb. 26, 1938			Remarks
		Larvae	Pupae	Adult	
1	48	33	10	5	50 larvae exposed to +20°F. on Feb. 11. 4% mortality. Brood dead on Feb. 26 from mold.
2	31	5	6	20	50 larvae exposed to +15°F. on Feb. 11. 38% mortality. Brood dead on Feb. 26 from mold.
3	19	11	5	3	50 larvae exposed to +10°F. on Feb. 11. 62% mortality. Brood dead on Feb. 26 from mold.

It is recognized that if a more careful technique had been employed in testing the subsequent development of exposed larvae, a greater number would undoubtedly have reached maturity, and that life might have been maintained for a longer period. However, these tests served their purpose of demonstrating the possibilities of the experiment,

and it would seem that a large percent of the more resistant larvae are capable of developing to new adults following an exposure to a near lethal temperature.

SUMMARY OF 1937-1938 EXPERIMENTS

1. Bark-beetle larvae can be stored at a temperature of 38° - 40°F. for a period of 60 or more days with no mortality. Larvae should be placed in petri dishes with a small piece of saturated blotting paper attached to the inside of the petri dish lid to provide additional moisture.

2. Bark-beetle mortality associated with an exposure to low temperatures depends upon the severity of the temperature and not upon the length of the exposure. Bark-beetle larvae were exposed to a set temperature for 10 days with no increased mortality.

3. During the winter of 1937-1938 the resistance or cold-hardiness of mountain pine beetle larvae increased in early September and built quite rapidly with the approach of winter temperatures until a maximum resistance was reached in early December. With the occurrence of warm weather during the latter part of February the resistance dropped quite rapidly, reaching its low point during the middle of May. Subsequent to that date the resistance increased somewhat as the over-wintering larvae reached the prepupal and pupal stages of development. As the equipment available at this laboratory will only provide a minimum temperature of -25°F., it has been necessary to consider the ability of the larvae to withstand an exposure to this temperature as the maximum larval resistance. It is recognized that were lower temperatures available our studies would have shown an increased resistance, though it is believed that there is a period of several weeks, where the resistance remains quite constant.

4. The cold-hardiness of overwintering bark-beetle larvae to low temperatures can be broken by an exposure to activity temperatures. The degree to which the resistance will be lowered depends upon the length of exposure.

5. Larvae can be stored for a period of 10 days at a temperature of 35° - 40°F. with no reduction in their resistance to low temperatures.

CONCLUSIONS

As a result of experiments conducted during the past two seasons, it is apparent that mountain pine beetle larvae in white pine when subjected to the occurrence of normal fall and winter temperatures become resistant to minimum winter temperatures. However, the occurrence of abnormal low temperatures before the larvae have been adequately tempered or in the spring after cold resistance has been broken, or the occurrence of abnormal warm weather during a period of maximum resistance is believed to be responsible for excessive larval mortality often recorded in overwintering broods of the mountain pine beetle. The occurrence of low temperatures in early fall or spring seasons as well as unusually warm temperatures during winter months should be considered as being potentially beneficial in destroying overwintering bark-beetle broods.

THE RELATION OF LIPID AND MOISTURE CONTENT
TO COLD-HARDINESS OF MOUNTAIN PINE BEETLE LARVAE

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By
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Associate Entomologist

During the course of the low-temperature work at the Coeur-d'Alene laboratory the fact has been well established that even when mountain pine beetle larvae have been tempered under identical circumstances there is a considerable variation in their resistance to cold. This led to a series of tests to determine the effect of the lipid-moisture relationship on the cold-hardiness of these insects. The theoretical basis for these tests as well as the technique used in making them was discussed in the 1937 report so that this memorandum will be restricted to a resume of the work done during the winter of 1937-38.

Table I shows the variation in the lipid-moisture relationship in resistant and non-resistant larvae. Moisture content is expressed as percent of live weight, while ether-soluble fat and oil content is shown for both live and dry weights. Since the amount of lipids in comparison to the amount of moisture is the important factor, if the theory is correct, this relationship is expressed as a ratio.

Table I

LIPID-MOISTURE CONTENT OF FROZEN AND UNFROZEN LARVAE
EXPOSED AT -20°F. FOR 24 HOURS

Condition of larvae	Moisture content	Lipid content: Dry weight	Lipid content: Live weight	Lipid-moisture ratio
	Percent	Percent	Percent	
Frozen	60.2	16.0	6.3	1/9.5
Unfrozen	62.1	34.6	13.1	1/4.7

The results of the test shown in table I correspond to the results secured from similar tests during the preceding winter. The lipid content of the unfrozen larvae was approximately twice as great as that of the frozen larvae, while the moisture content was nearly the same. This not only shows the difference in resistant and nonresistant larvae, but indicates the variation which can be expected in a single sample of larvae taken from the same log.

The apparent relationship between high lipid content and cold-hardiness is further shown in table II, which presents the results from periodic samples taken from September 18, 1937 to May 23, 1938. In this table the last column shows the approximate temperature at which 50-percent mortality occurred in samples taken from the same log.

Table II

SEASONAL VARIATION IN LIPID-MOISTURE CONTENT

Date of test	Moisture content	Lipid content: Dry weight	Lipid content: Live weight	Lipid-moisture ratio	Temperature at which 50% mortality occurred
	Percent	Percent	Percent		°F.
9/18/37	64.8	20	7	1/9.2	12.5
10/9/37	60	23	9.1	1/6.5	9
10/22/37	57.7	26.9	11.3	1/5.1	1
11/10/37	54	23.5	10.7	1/5.0	-14
11/29/37	54.6	24.9	11.3	1/4.8	-27
12/7/37	58.3	24.3	10.1	1/5.7	No kill
12/27/37	68.4	23.6	9	1/7.6	
1/7/38	66.8	28.9	9.5	1/7	
1/31/38	65.4	33.5	11.6	1/5.6	
2/7/38	61.2	30.6	11.9	1/5.1	
2/10/38	66.6	40.1	13.4	1/4.9	
3/7/38	63.6	32.9	11.9	1/5.3	-18.5
3/22/38	62.7	29.1	11	1/5.7	- 5.5
4/4/38	66.2	28.9	9.7	1/6.8	-12
4/11/38	64.4	23.1	8.2	1/7.8	10
4/25/38	68.8	26.6	8.3	1/8.2	13
5/2/38	69.7	29.5	8.9	1/7.7	17.25
5/9/38	70.1	29.6	8	1/8.7	13
5/23/38	65.7	17.6	6.3	1/10.4	18

Although table II shows a general tendency toward an increase in lipid content during the colder months of the year, there are some irregularities which undoubtedly result from variations in the different samples taken from different logs. It is to be noted also in this table that in general there was less resistance to cold by those larvae with a large denominator in the lipid-moisture ratio. There are, however, some samples which do not agree with this general conclusion. In explanation of these variations, the technique employed was undoubtedly the cause. It has already been explained that there is considerable variation in individual larvae, so that the extracted sample may not

have been representative. In addition, the mortality temperature was secured from a smoothed-out curve plotted from a maximum of eight samples which gives two more possibilities of error. First, any one of the samples used in determining the curve may not have been representative, and second, the revised curve does not show the actual resistance of a sample comparable to the one extracted.

In general, however, the correlation is sufficiently well-defined to indicate a close relationship between lipid-moisture content and cold-hardiness. The approach to this resistant condition is an important consideration in determining the possibilities for mortality to overwintering broods. To secure some information on tempering, three experiments were performed as shown in tables III, IV, and V. In the tests shown in table III logs were brought from field storage and the larvae exposed or extracted as shown. In table IV the logs brought from field storage were held ten days at room temperature until the larvae were active and feeding. An attempt was then made to temper these larvae by various exposures in the refrigerator. Table V shows the results from a similar test in which the larvae were left in the log and allowed to temper by field storage for different intervals.

Table III

EFFECT OF COOLING ON LIPID-MOISTURE CONTENT OF MOUNTAIN PINE BEETLE LARVAE

Treatment	Moisture content	Lipid content: Dry weight	Lipid content: Live weight	Lipid-moisture ratio
	Percent	Percent	Percent	
Stored in refrigerator 24 hours	64.4	22.6	8	1/8.0
Extracted upon removal from log	65.3	17	5.8	1/11.2
Stored in refrigerator 24 hours	59.3	23	9.3	1/6.3
Extracted upon removal from log	59.8	23	9.1	1/6.5
Stored in refrigerator 24 hours	57.3	28	11.9	1/4.8
Extracted upon removal from log	58.1	25.7	10.7	1/5.4
Stored in refrigerator 14 days	54	23.5	10.7	1/5.0
Extracted upon removal from log	55.2	23.4	10.2	1/5.4
Stored 10 days at -20°F.	64.7	33.4	11.8	1/5.4
Stored 1 day at -20°F.	67.8	40	12.8	1/5.2
Extracted upon removal from log	68.4	23.6	9	1/7.6
Stored 10 days at -25°F.	65	35.2	12.2	1/5.3
Stored 1 day at -25°F.	56.3	41.4	18	1/3.1
Untreated	66.8	28.9	9.5	1/7

Table IV

RESULT OF ARTIFICIAL HARDENING IN REFRIGERATOR

Treatment [a]	Moisture content	Lipid content: Dry weight	Lipid content: Live weight	Lipid-moisture ratio	Mortality at +5°F.
	Percent	Percent	Percent		Percent
Sample taken when logs brought from field	66.6	40.1	13.4	1/4.97	0
Sample taken after exposure at room temp. 10 days	70.9	22.8	6.6	1/10.74	96
32°F. for 24 hours	68.8	32.2	10	1/6.88	
32°F. for 7 days	68.2	34.2	10.8	1/6.31	
32°F. for 14 days	61.3	39.1	15.1	1/4.05	
32°F. for 30 days	64.1	38	13.6	1/4.71	12
40°F. for 24 hours	67.9	25.3	8.1	1/8.38	
40°F. for 7 days	65.9	26.9	9.3	1/7.08	
40°F. for 14 days	64.9	29.5	10.3	1/6.30	
40°F. for 30 days	66.4	37.8	12.7	1/5.22	42
Alternate 24-hour periods at room temp. and 40°F. for 2 weeks	70.1	30.9	9.2	1/7.61	
Alternate 24-hour periods at room temp. and 32°F. for 2 weeks	70.6	33.1	9.7	1/7.27	58

[a] Logs used in these tests were brought from field storage to room storage and held for 10 days.

Table V

RESULT OF HARDENING UNDER FIELD CONDITIONS

Test	Treatment	Moisture content	Lipid content: Dry weight	Lipid content: Live weight	Lipid-moisture ratio	Temperature at which 50% mortality occurred
		Percent	Percent			°F.
X1a	Sample taken when log brought from field storage to room storage	65.4	33.5	11.6	1/5.6	
X1b	Sample taken after log stored at room temperature 7 days	66.7	29.7	9.9	1/6.7	13.75
X2	Sample taken after log returned to field storage for 3 days	66.6	24.6	8.2	1/8.1	12.5
X3	Sample taken after log returned to field storage for 12 days	68.5	36.4	11.4	1/6.0	4
XX1a	Sample taken when log brought from field storage to room storage	61.2	30.6	11.9	1/5.1	
XX1b	Sample taken after log stored at room temperature 3 days	61.3	27	10.4	1/6.0	2.5
XX2	Sample taken after log returned to field storage 9 days	64.9	44	15.4	1/4.2	-6
XX3	Sample taken after log returned to field storage 25 days	64.2	24.2	8.6	1/7.4	-5.25
XXX1a	Sample taken when log brought from field storage to room storage	68.8	29.7	9.2	1/7.4	
XXX1b	Sample taken after log stored at room temperature 2 days	63.3	33.6	12.3	1/5.1	3

Table V (Cont.)

Test	Treatment	Moisture content	Lipid content Dry weight	Lipid content Live weight	Lipid-moisture ratio	Temperature at which 50% mortality occurred
		Percent	Percent			°F.
XXX2	Sample taken after log returned to field storage 14 days	63.2	27.9	10.2	1/6.1	-6.5
XXXX1	Sample taken after log brought from field storage to unheated garage 4 days	64.8	38.4	13.5	1/4.6	-19
XXXX3	Sample taken after log stored in garage 14 days	64.1	24.1	8.6	1/7.4	

Table III shows that when larvae are exposed to a temperature below that at which they have been stored, there is a decided increase in the lipid-moisture ratio, provided the temperature is not too severe so as to cause mortality. This would indicate that the larvae are capable of independent fat and oil synthesis upon subjection to mild shocks of low temperature.

Table IV shows that prolonged exposures at set temperatures tend to build up the lipid content of larvae, but exposure of the check samples at +5°F. indicated that cold-hardiness of the larvae had not been returned to the original condition even though the lipid-moisture ratios were comparable. This may have been caused by the prolonged holding of the larvae out of their native habitat, or it may indicate that there are other factors involved in tempering which were not con-

sidered. It may be that larvae are capable of voluntary or involuntary synthesis of fats and oils only after they have reached a certain point in the tempering process. In the tests shown in table IV the larvae were taken from an active feeding condition and subjected to temperatures which soon produced dormancy. Under field conditions, the temperature change in the fall is much more gradual and it is possible that the larvae perform a certain amount of selective feeding under the stimulus of decreasing temperatures.

The results shown in table V, in which some attempt was made to determine the effect of field tempering, are not satisfactory because here again the change in temperature was too abrupt and the accuracy of the sampling leaves much to be desired.

In general there is an apparent correlation between the lipid-moisture content of mountain pine beetle larvae and their resistance to low temperatures. However, owing to the technique of sampling, this relationship is not clearly shown in tests discussed in this memorandum.